SUPPLEMENTAL DATA

FIGURE LEGENDS TO SUPPLEMENTARY DATA

Figure S1. Wsc1p variants are misfolded. (A) Schematic representation of HA-tagged Wsc1p and its variants. The indicated mutations are marked by asterisks. S.S., signal sequence; TM, transmembrane domain; Cyt, cytoplasmic domain. (B) Pulse-chase analysis was performed in wild type and *sec12-4* strains expressing Wsc1p and Δ68-80 as described in Figure 1B. (C) The folding status of Wsc1p and its variants were examined by a PEGylation-based folding assay as described in Figure 1D.

Figure S2. Degradation of Wsc1p variants is ERAD independent. (A-E) The turnover rates of Wsc1p variants in WT, Δ*cue1*, Δ*hrd1* and Δ*doa10* cells were examined by pulse-chase analysis as described in Figure 2A.

Figure S3. Wsc1-Δ68-80 is stable when retained in the ER. Stability of Wsc1-Δ68-80 was examined in wild type and *sec12-4* strains using pulse-chase analysis as described in Figure 3A.

Figure S4. Wsc1p variants degrade in the vacuole. (A-B) Pulse-chase analysis was performed to examine the degradation of Wsc1p variants in wild type and Δ*pep4* cells as described in Figure 2A.

Figure S5. Visualization of the vacuolar ATPase by indirect immunofluorescence. Wild type cells were processed as described in Figure 3D and stained with anti-V-ATPase (60 kDa subunit) primary antibodies followed by Alexa Fluor 546 goat anti-mouse secondary antibodies. The cells

were visualized by confocal and DIC microscopy. Note that V-ATPase staining coincides with structures that appear as optical depressions using DIC. Scale bar, 5 μm.

Figure S6. Degradation of Wsc1-L63R is autophagy independent. (A) Wild type and Δ*apg8* cells expressing Wsc1-L63R were analyzed by pulse-chase analysis as described in Figure 2A. (B) Wsc1-L63R turnover was analyzed by pulse-chase analysis in wild type and Δvps10 cells as described in Figure 2A.

Figure S7. Wsc1-Δ68-80 does not contain an ERAD determinant in the luminal domain. (A) A schematic diagram of Wsc1-Δ68-80 and Wsc1-Δ68-80$_{Luminal}$. S.S., signal sequence; TM, transmembrane domain; Cyt, cytoplasmic domain. (B) The localization of Wsc1-Δ68-80$_{Luminal}$ was examined by indirect immunofluorescence as described in Figure 5B. Scale bar, 5 μm. (C) The turnover of Wsc1-Δ68-80 and Wsc1-Δ68-80$_{Luminal}$ in the wild type strain was measured by pulse-chase analysis as described in Figure 2A. (D) Pulse-chase analysis was performed in wild type and *sec12-4* strains expressing Wsc1-Δ68-80$_{Luminal}$ as described in Figure 1B.

Figure S8. Wsc1-Δ68-80 fused with an ERAD determinant is recognized by ERAD. (A) Schematic representation of ED-Wsc1-Δ68-80 and ED-Wsc1-Δ68-80$_{Luminal}$. The CPY C-terminal domain contains a single carbohydrate and is represented by a branched symbol. S.S., signal sequence of Kar2p; CPY CTD, the CPY C-terminal domain; TM, transmembrane domain; Cyt, cytoplasmic domain. (B) The turnover of ED-Wsc1-Δ68-80 was examined in wild type and various mutants using pulse-chase analysis as described in Figure 2A. (C) Wild type and mutant

cells expressing ED-Wsc1-Δ68-80$_{Luminal}$ were analyzed by pulse-chase analysis as described in Figure 2A.

SUPPLEMENTARY TABLES

Table S1. Strains used in this study

Strain	Genotype	Source
W303	*Mat***a**, *leu2-3*, *112*, *his3-11*, *trp1-1*, *ura3-1*, *can1-100*, *ade2-1*	P. Walter (UCSF)
BY4742	*Matα*, *leu2Δ0*, *lys2Δ0, his3Δ1*, *ura3Δ0*	Research Genetics, Huntsville, AL
ESY258	*Mat***a**, pDN436, W303 background	Spear and Ng, 2003
SWY300	*Mat***a**, pRS315, W303 background	This study
SWY330	*Mat***a**, pSW100, W303 background	This study
SWY382	*Mat***a**, *sec12-4*, pSW100, W303 background	This study
SWY338	*Mat***a**, pSW102, W303 background	This study
SWY383	*Mat***a**, *sec12-4*, pSW102, W303 background	This study
SWY339	*Mat***a**, *cue1::TRP1*, pSW102, W303 background	This study
SWY340	*Mat***a**, *hrd1::KANMX*, pSW102, W303 background	This study
SWY614	*Mat***a**, *doa10::KANMX*, pSW102, W303 background	This study
SWY341	*Mat***a**, *pep4::HIS3*, pSW102, W303 background	This study
SWY342	*Mat***a**, pSW104, W303 background	This study
SWY384	*Mat***a**, *sec12-4*, pSW104, W303 background	This study
SWY343	*Mat***a**, *cue1::TRP1*, pSW104, W303 background	This study
SWY344	*Mat***a**, *hrd1::KANMX*, pSW104, W303 background	This study
SWY367	*Mat***a**, *doa10::KANMX*, pSW104, W303 background	This study
SWY345	*Mat***a**, *pep4::HIS3*, pSW104, W303 background	This study
SWY416	*Mat***a**, *vps10::KANMX*, pSW104, W303 background	This study
SWY653	*Mat***a**, *ktr1::KANMX*, *ktr3::KANMX*, *kre2::TRP1*, pSW104, W303 background	This study
SWY402	*Matα*, pSW104, BY4742 background	This study
SWY403	*Matα*, *apg8:: KANMX*, pSW104, BY4742 background	This study
SWY346	*Mat***a**, pSW105, W303 background	This study
SWY385	*Mat***a**, *sec12-4*, pSW105, W303 background	This study
SWY347	*Mat***a**, *cue1::TRP1*, pSW105, W303 background	This study
SWY348	*Mat***a**, *hrd1::KANMX*, pSW105, W303 background	This study
SWY615	*Mat***a**, *doa10::KANMX*, pSW105, W303 background	This study
SWY349	*Mat***a**, *pep4::HIS3*, pSW105, W303 background	This study
SWY354	*Mat***a**, pSW112, W303 background	This study
SWY387	*Mat***a**, *sec12-4*, pSW112, W303 background	This study
SWY355	*Mat***a**, *cue1::TRP1*, pSW112, W303 background	This study
SWY356	*Mat***a**, *hrd1::KANMX*, pSW112, W303 background	This study
SWY369	*Mat***a**, *doa10::KANMX*, pSW112, W303 background	This study

SWY357	*Mat***a**, *pep4::HIS3*, pSW112, W303 background	This study
SWY358	*Mat***a**, pSW113, W303 background	This study
SWY388	*Mat***a**, *sec12-4*, pSW113, W303 background	This study
SWY359	*Mat***a**, *cue1::TRP1*, pSW113, W303 background	This study
SWY360	*Mat***a**, *hrd1::KANMX*, pSW113, W303 background	This study
SWY371	*Mat***a**, *doa10::KANMX*, pSW113, W303 background	This study
SWY361	*Mat***a**, *pep4::HIS3*, pSW113, W303 background	This study
SWY420	*Mat***a**, *vps10::KANMX*, pSW113, W303 background	This study
SWY362	*Mat***a**, pSW114, W303 background	This study
SWY389	*Mat***a**, *sec12-4*, pSW114, W303 background	This study
SWY363	*Mat***a**, *cue1::TRP1*, pSW114, W303 background	This study
SWY364	*Mat***a**, *hrd1::KANMX*, pSW114, W303 background	This study
SWY376	*Mat***a**, *doa10::KANMX*, pSW114, W303 background	This study
SWY365	*Mat***a**, *pep4::HIS3*, pSW114, W303 background	This study
SWY423	*Mat***a**, pSW119, W303 background	This study
SWY508	*Mat***a**, *sec12-4*, pSW119, W303 background	This study
SWY458	*Mat***a**, pSW122, W303 background	This study
SWY511	*Mat***a**, *sec12-4*, pSW122, W303 background	This study
SWY541	*Mat***a**, pSW144, W303 background	This study
SWY543	*Mat***a**, *cue1::TRP1*, pSW144, W303 background	This study
SWY544	*Mat***a**, *hrd1::KANMX*, pSW144, W303 background	This study
SWY542	*Mat***a**, *pep4::HIS3*, pSW144, W303 background	This study
SWY561	*Mat***a**, *pep4::HIS3*, *cue1::TRP1*, pSW144, W303 background	This study
SWY562	*Mat***a**, *pep4::HIS3*, *hrd1::KANMX*, pSW144, W303 background	This study
SWY545	*Mat***a**, pSW145, W303 background	This study
SWY547	*Mat***a**, *cue1::TRP1*, pSW145, W303 background	This study
SWY548	*Mat***a**, *hrd1::KANMX*, pSW145, W303 background	This study
SWY546	*Mat***a**, *pep4::HIS3*, pSW145, W303 background	This study
SWY564	*Mat***a**, *pep4::HIS3*, *cue1::TRP1*, pSW145, W303 background	This study
SWY565	*Mat***a**, *pep4::HIS3*, *hrd1::KANMX*, pSW145, W303 background	This study
SWY579	*Mat***a**, pSW153, W303 background	This study
SWY581	*Mat***a**, *cue1::TRP1*, pSW153, W303 background	This study
SWY582	*Mat***a**, *hrd1::KANMX*, pSW153, W303 background	This study
SWY580	*Mat***a**, *pep4::HIS3*, pSW153, W303 background	This study
SWY587	*Mat***a**, pSW155, W303 background	This study
SWY589	*Mat***a**, *cue1::TRP1*, pSW155, W303 background	This study
SWY590	*Mat***a**, *hrd1::KANMX*, pSW155, W303 background	This study
SWY588	*Mat***a**, *pep4::HIS3*, pSW155, W303 background	This study
SWY601	*Mat***a**, pSW156, W303 background	This study
SWY603	*Mat***a**, pSW157, W303 background	This study
SWY604	*Mat***a**, *pep4::HIS3*, pSW157, W303 background	This study
SWY605	*Mat***a**, pSW158, W303 background	This study
SWY606	*Mat***a**, *pep4::HIS3*, pSW158, W303 background	This study

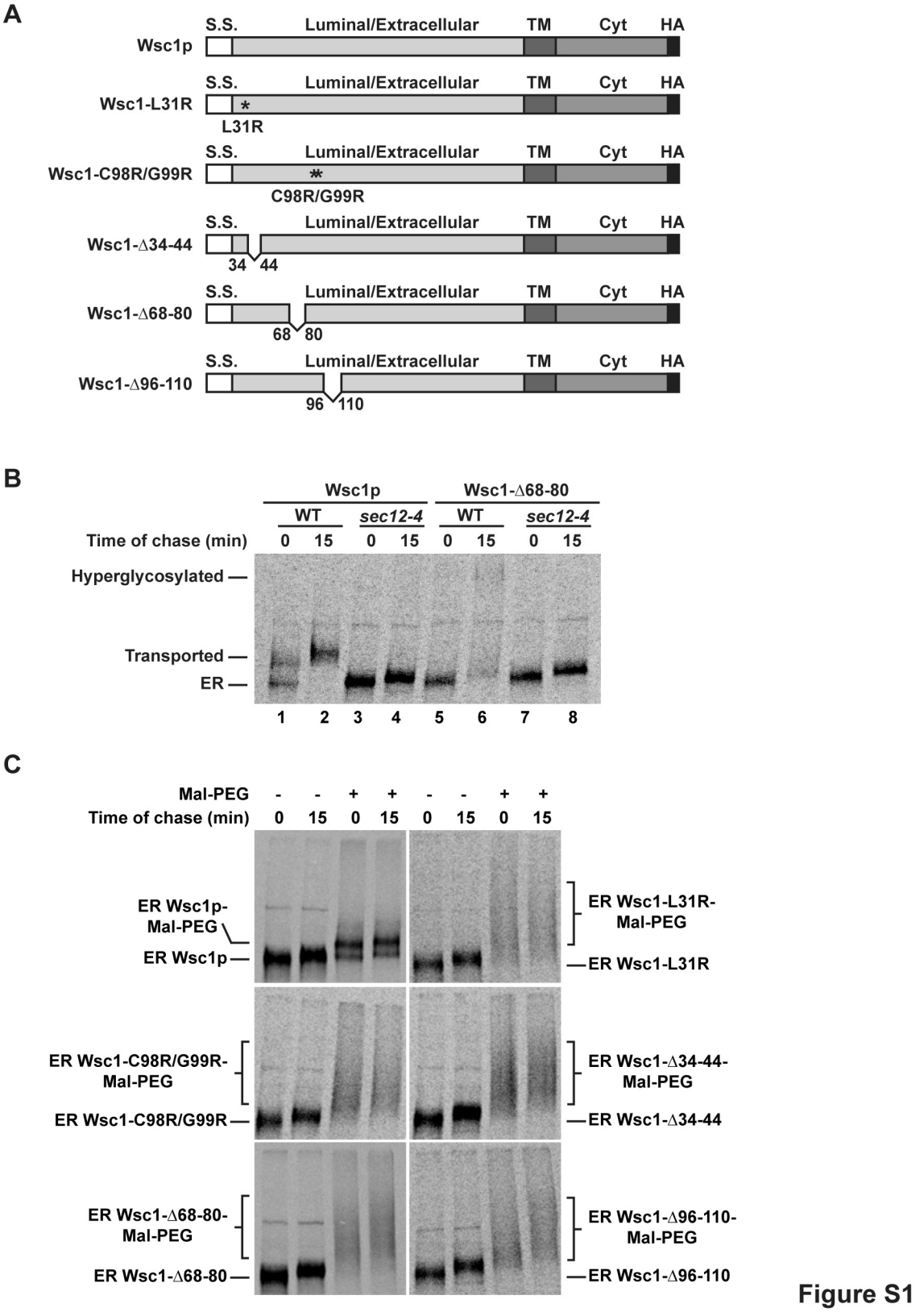
Table S2. Plasmids modified by site-directed mutagenesis

Plasmid	Protein	Primer	Mutation	Template	Vector	Source
pSW102	Wsc1-L31R	SWN64	L31R	pSW100	pRS315	This study
pSW104	Wsc1-L63R	SWN66	L63R	pSW100	pRS315	This study
pSW105	Wsc1-C98R/ G99R	SWN67	C98R, G99R	pSW100	pRS315	This study
pSW112	Wsc1-Δ34-44	SWN72	Deletion of D34 to Q44	pSW100	pRS315	This study
pSW113	Wsc1-Δ68-80	SWN73	Deletion of E68 to E80	pSW100	pRS315	This study
pSW114	Wsc1-Δ96-110	SWN74	Deletion of to E96 to D110	pSW100	pRS315	This study
pSW119	Wsc1-L63R $_{\text{Luminal}}$	SWN76	Deletion of DNA TMD and Cytoplasmic domains of Wsc1-L63R (Deletion of V262 to D378)	pSW104	pRS315	This study
pSW122	Wsc1-Δ68-80 $_{\text{Luminal}}$	SWN76	Deletion of DNA TMD and cytoplasmic domains of Wsc1 Δ68-80	pSW113	pRS315	This study
pSW153	ED-Wsc1-L63R$_{\text{Luminal}}$	SWN76	Deletion of DNA TMD and cytoplasmic domains of ED-Wsc1-L63R	pSW144	pRS315	This study
pSW155	ED-Wsc1-Δ68-80$_{\text{Luminal}}$	SWN76	Deletion of DNA TMD and cytoplasmic domains of ED-Wsc1-Δ68-80	pSW145	pRS315	This study
pSW156	Wsc1-AAA	SWN77	N344A, P345A, F346A	pSW147	pRS315	This study
pSW157	Wsc1-L63R-AAA	SWN77	N344A, P345A, F346A	pSW148	pRS315	This study
pSW158	Wsc1-Δ68-80-AAA	SWN77	N331A, P332A, F333A	pSW149	pRS315	This study

Table S3. Oligonucleotide primers used in this study

Primer	Construct	Sequence (5'→3')

SWN1	pSW3	CGCGGATCCATGAGACCGAACAAAACAAGTC
SWN2	pSW3	CTCATGCCATGGCATCAGCTTCGTCTGGATTGACC
SWN50	pSW144, pSW145	GTTTCCCGACTGGAAAGCGGGCAGTG
SWN64	pSW102	GAATACGTGAATTGTTTTAGCTCAAGACCCTCTGACTTTTCAAAGGCCGA
SWN66	pSW104	CGCAAAAGGTGCAAGCTACTTTGCCAGATATAATCATTCAGAATGTTATTGTG
SWN67	pSW105	CTTTGGTTACAGCAGTGAGATGTGTAGAAGAGAAGATGCCTATTCTGTGTACCAAC
SWN72	pSW112	GAATTGTTTTAGCTCACTACCCTCTTCGAGTTCACACTGTAACAGTGAG
SWN73	pSW113	CTACTTTGCCCTTTATAATCATTCATCTACTTCCTCTTCATGTAATACG
SWN74	pSW114	ATACGTATTGCTTTGGTTACAGCAGTTCTGACACAAATAGCAATAGCATAAG
SWN76	pSW119, pSW122, pSW153, pSW155	GTAAAACTCATAAAAAGAAAGCCAATGCCATGGCCTACCCATATGATGTTC
SWN77	pSW156, pSW157, pSW158	GCACACCAAAGGGCAAACTGATATTGCCGCCGCCGACGACTCAAGGAGAATAAGTA ACG
SWN84	pSW144, pSW145	GGAAAAAAGCGGCCGCAATTCCGTATATGATGATACATATGTTAGGTC
SWN85	pSW144, pSW145	TAAGGAGAAACCACCGTGGATCCATTCG
SWN86	pSW144, pSW145	TATGAATACGTGAATTGTTTTAGCTCACTACC
SWN87	pSW147, pSW148, pSW149	ATAAGAATGCGGCCGCGGAAGGCACCCTTTTCGAAGG
SWN88	pSW147, pSW148, pSW149	ACATGGATCCTGTTGAGATTTAGCTGTGTTTGTTG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Luminal/Extracellular
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Cyt
HA
Wsc1p
Wsc1-L31R
L31R
Wsc1-C98R/G99R
C98R/G99R
Wsc1-Δ34-44
34
44
Wsc1-Δ68-80
68
80
Wsc1-Δ96-110
96
110
B
Wsc1p
Wsc1-Δ68-80
WT
sec12-4
Time of chase (min)
0
15
Hyperglycosylated
Transported
ER
1
2
3
4
5
6
7
8
C
Mal-PEG
Time of chase (min)
ER Wsc1p-Mal-PEG
ER Wsc1p
ER Wsc1-L31R-Mal-PEG
ER Wsc1-L31R
ER Wsc1-C98R/G99R-Mal-PEG
ER Wsc1-C98R/G99R
ER Wsc1-Δ34-44-Mal-PEG
ER Wsc1-Δ34-44
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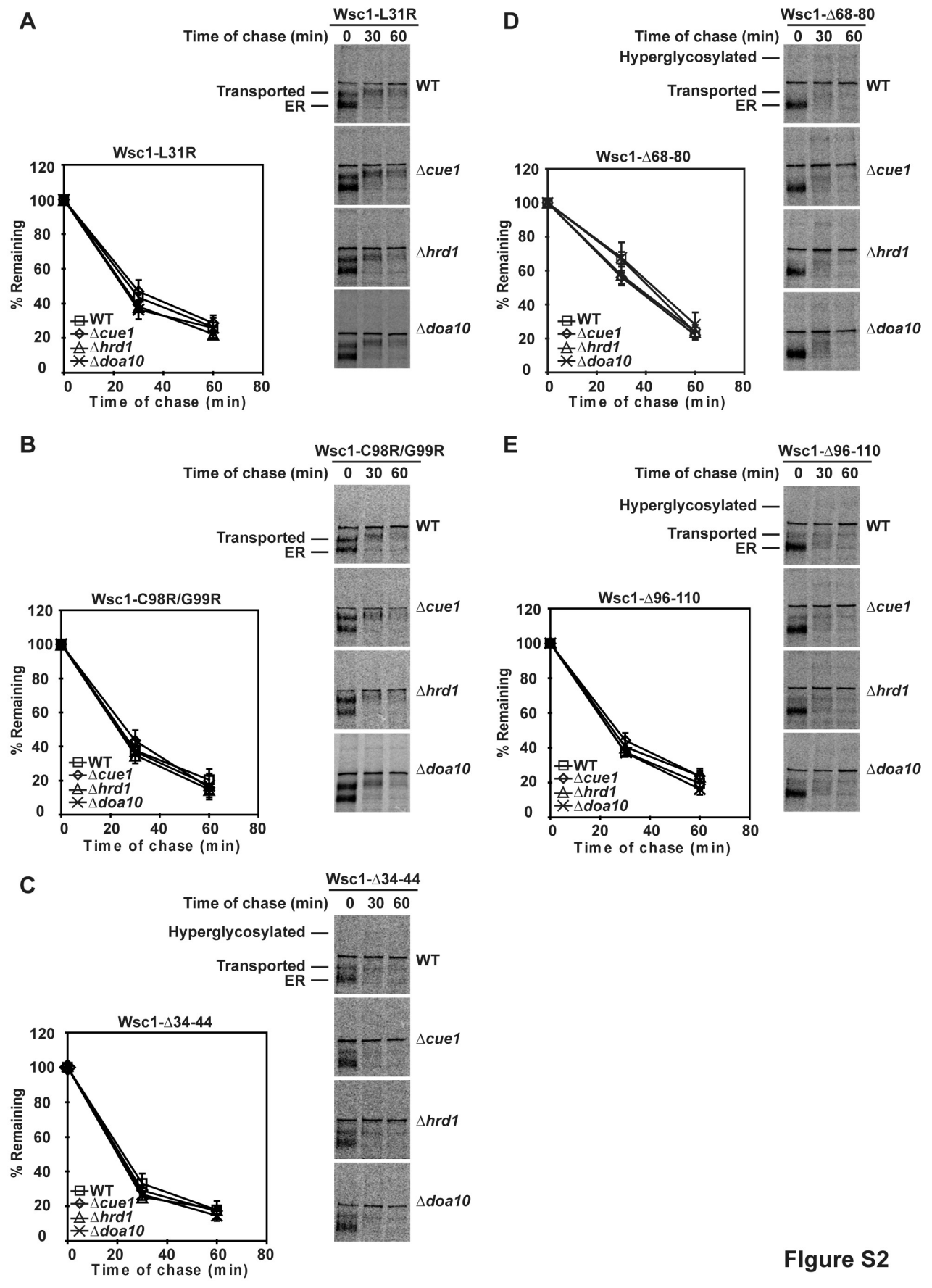
ER Wsc1-Δ96-110-Mal-PEG
ER Wsc1-Δ96-110

Figure S1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Time of chase (min)
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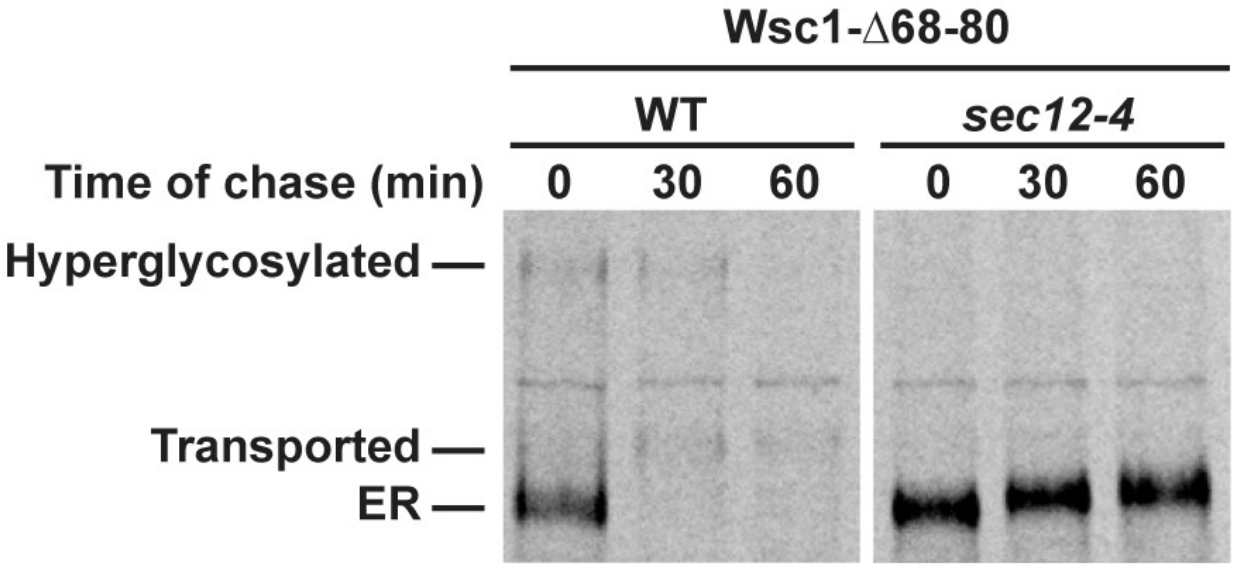
Transported
ER
WT
Δcue1
Δhrd1
Δdoa10
Wsc1-L31R
% Remaining
Time of chase (min)
WT
Δcue1
Δhrd1
Δdoa10
B
Wsc1-C98R/G99R
Time of chase (min)
0 30 60
Transported
ER
WT
Δcue1
Δhrd1
Δdoa10
Wsc1-C98R/G99R
% Remaining
Time of chase (min)
C
Wsc1-Δ34-44
Time of chase (min)
0 30 60
Hyperglycosylated
Transported
ER
WT
Δcue1
Δhrd1
Δdoa10
Wsc1-Δ34-44
% Remaining
Time of chase (min)
D
Wsc1-Δ68-80
Time of chase (min)
0 30 60
Hyperglycosylated
Transported
ER
WT
Δcue1
Δhrd1
Δdoa10
Wsc1-Δ68-80
% Remaining
Time of chase (min)
E
Wsc1-Δ96-110
Time of chase (min)
0 30 60
Hyperglycosylated
Transported
ER
WT
Δcue1
Δhrd1
Δdoa10
Wsc1-Δ96-110
% Remaining
Time of chase (min)

FIgure S2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sec12-4
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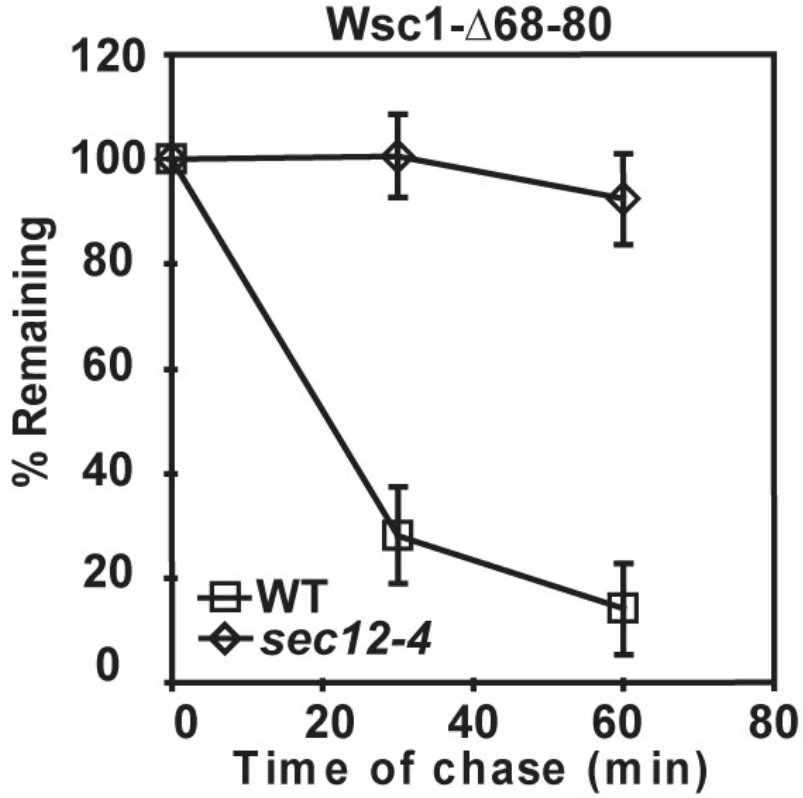
30
60
0
30
60
Hyperglycosylated
Transported
ER


Wsc1-Δ68-80
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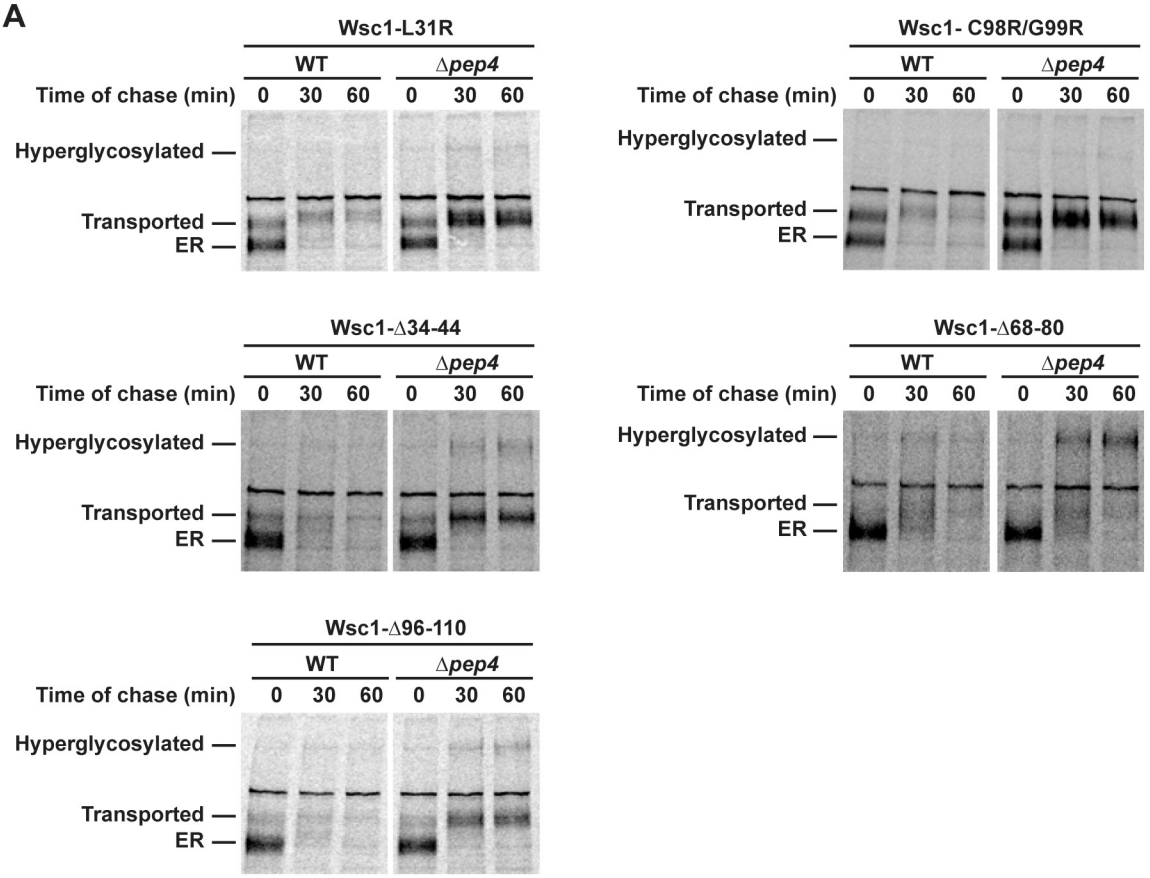
100
80
60
40
20
0
% Remaining
WT
sec12-4
0
20
40
60
80
Time of chase (min)

Figure S3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Wsc1-L31R
WT
Δpep4
Time of chase (min)
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Hyperglycosylated
Transported
ER
Wsc1- C98R/G99R
Wsc1-Δ34-44
Wsc1-Δ68-80
Wsc1-Δ96-110

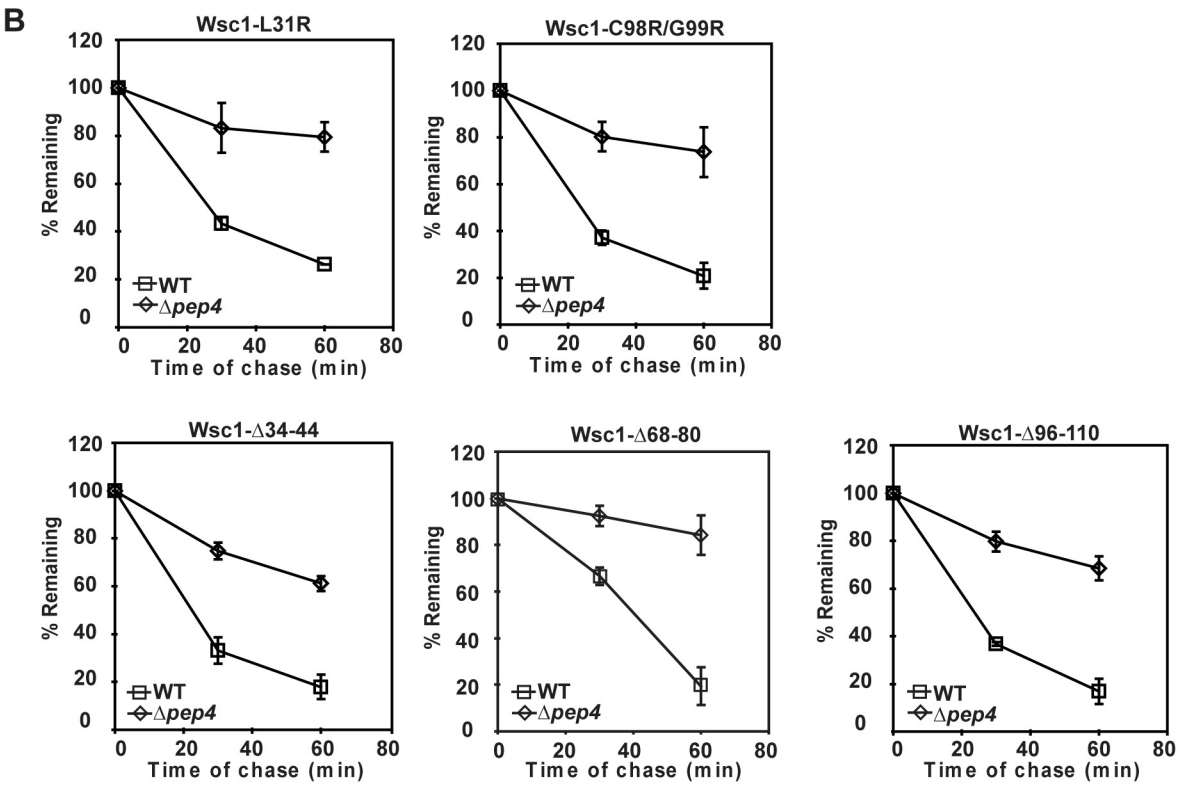

B
Wsc1-L31R
Wsc1-C98R/G99R
Wsc1-Δ34-44
Wsc1-Δ68-80
Wsc1-Δ96-110
% Remaining
Time of chase (min)
WT
Δpep4

Figure S4

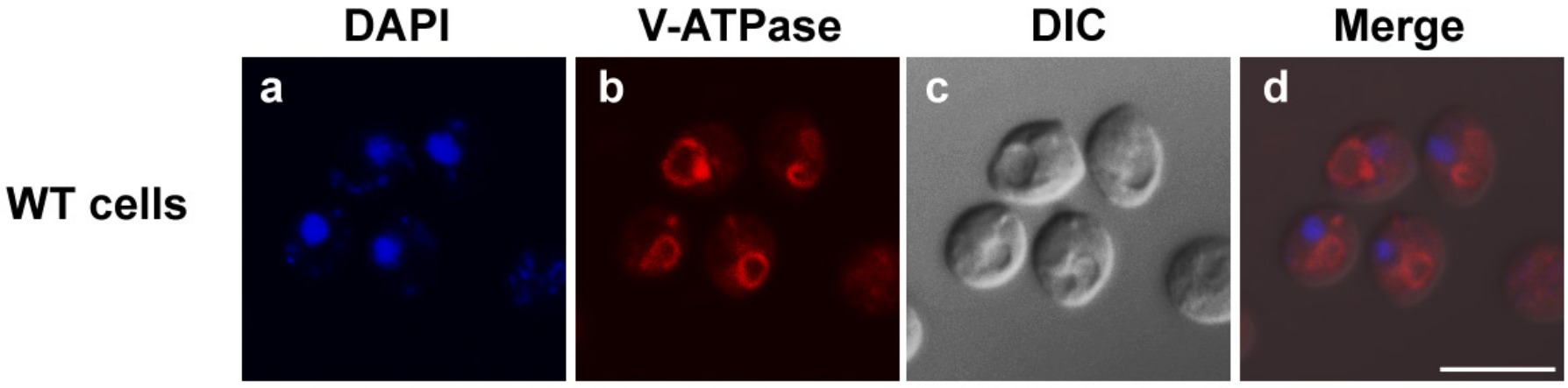
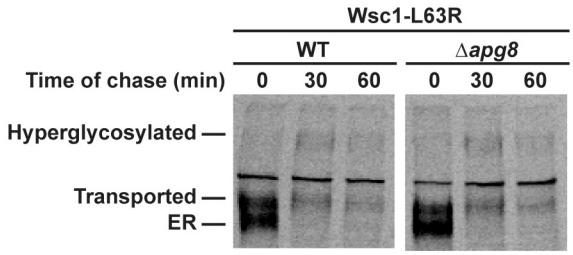


Figure S5

A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WT
Δapg8
Time of chase (min)
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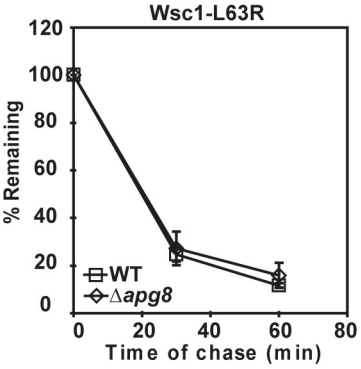
Hyperglycosylated
Transported
ER


Wsc1-L63R
% Remaining
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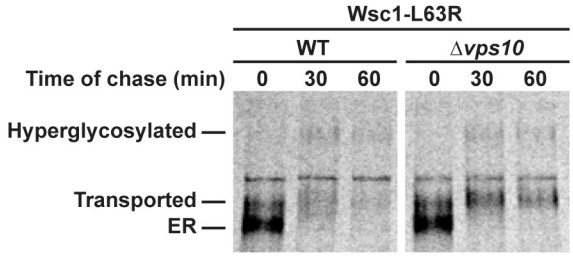
100
80
60
40
20
0
WT
Δapg8
0 20 40 60 80
Time of chase (min)

B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Δvps10
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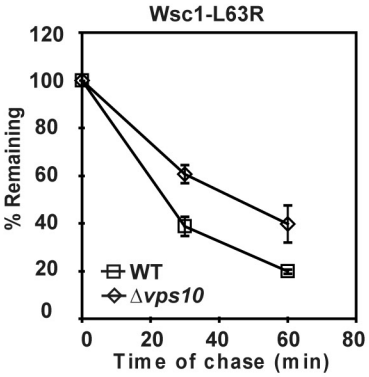
Hyperglycosylated
Transported
ER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% Remaining
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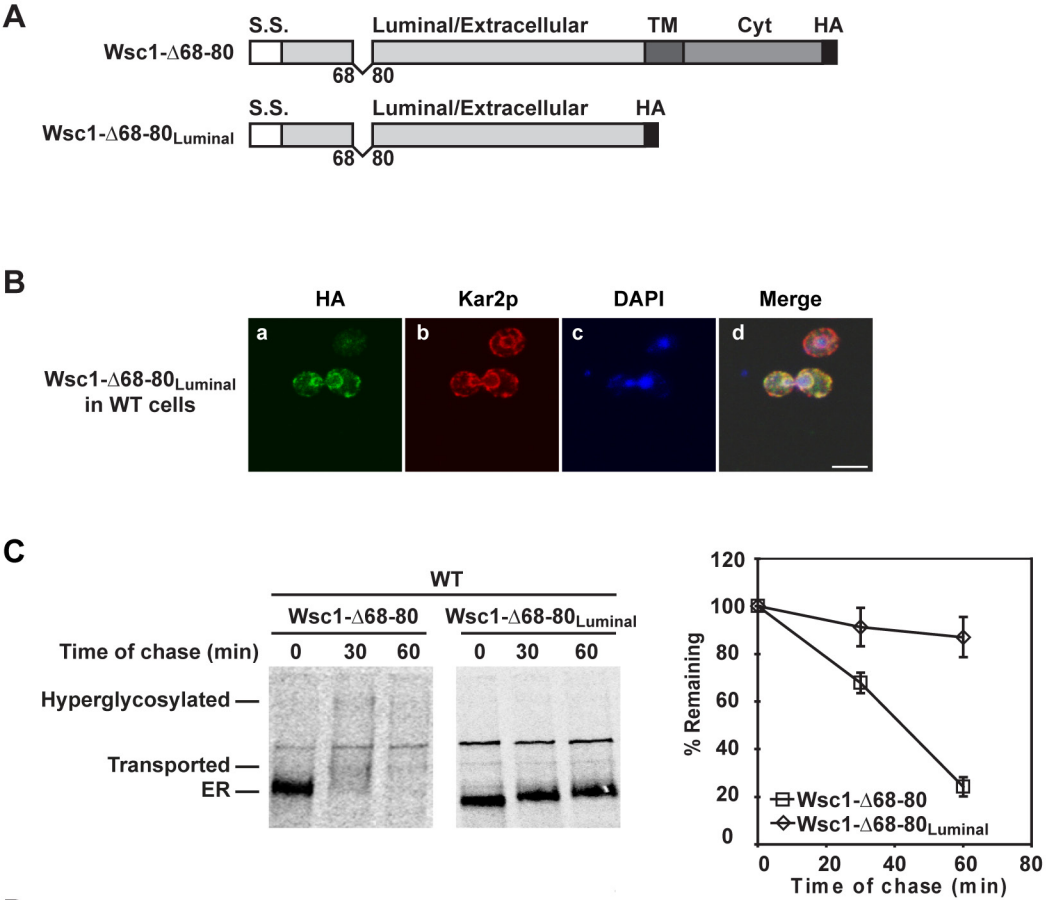
100
80
60
40
20
0
WT
Δvps10
0 20 40 60 80
Time of chase (min)

Figure S6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S.S.
Luminal/Extracellular
TM
Cyt
HA
Wsc1-Δ68-80
68
80
S.S.
Luminal/Extracellular
HA
Wsc1-Δ68-80Luminal
68
80
B
HA
Kar2p
DAPI
Merge
Wsc1-Δ68-80Luminal in WT cells
a
b
c
d
C
WT
Wsc1-Δ68-80
Wsc1-Δ68-80Luminal
Time of chase (min)
0
30
60
0
30
60
Hyperglycosylated
Transported
ER
120
100
80
60
40
20
0
% Remaining
Wsc1-Δ68-80
Wsc1-Δ68-80Luminal
0
20
40
60
80
Time of chase (min)
D
WT
sec12-4
Time of chase (min)
0
15
0
15
Wsc1-Δ68-80Luminal
1
2
3
4

Figure S7

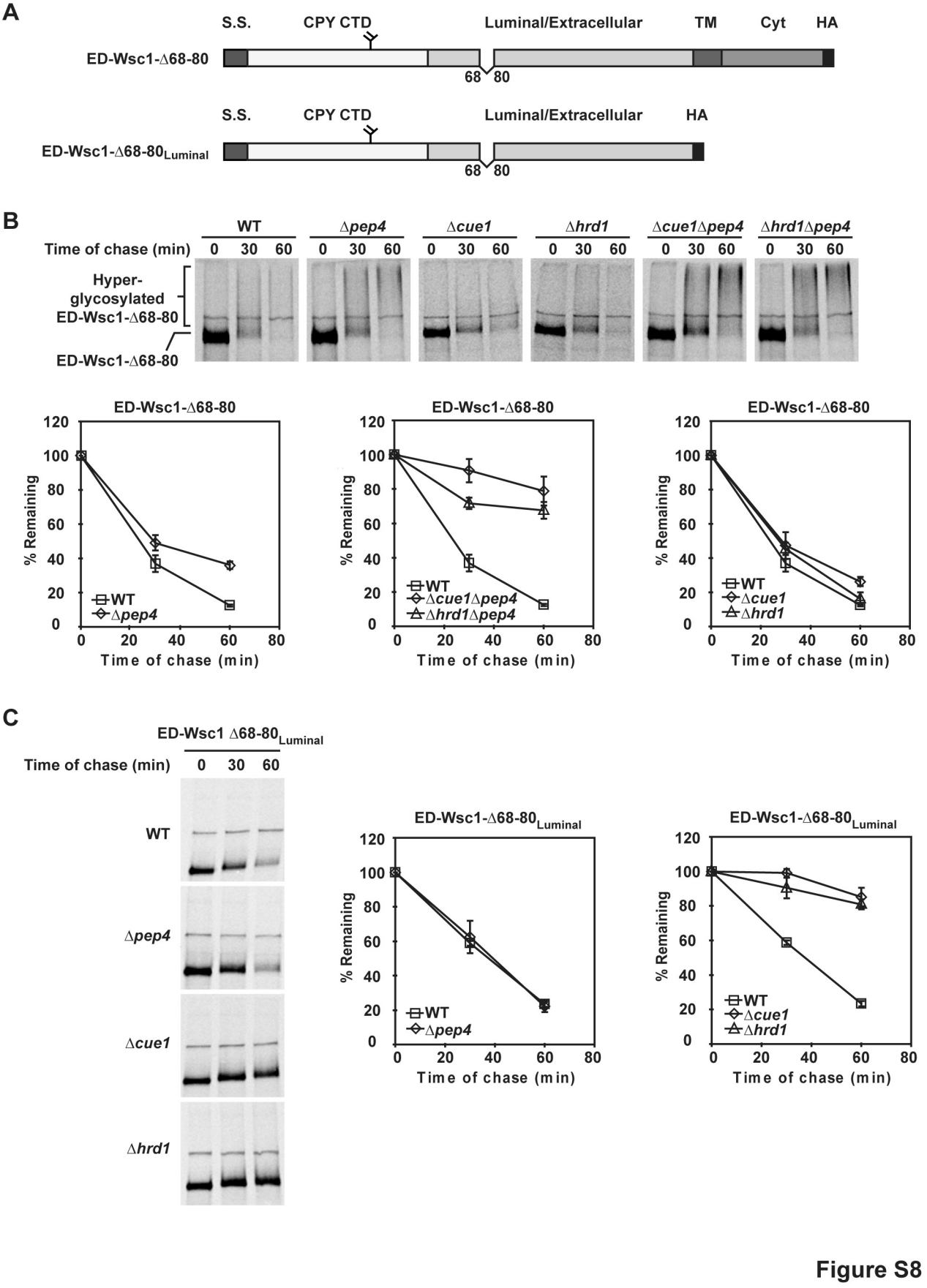
A
S.S.
CPY CTD
Luminal/Extracellular
TM
Cyt
HA
ED-Wsc1-Δ68-80
68
80
S.S.
CPY CTD
Luminal/Extracellular
HA
ED-Wsc1-Δ68-80Luminal
68
80
B
WT
Δpep4
Δcue1
Δhrd1
Δcue1Δpep4
Δhrd1Δpep4
Time of chase (min)
0 30 60
Hyper-glycosylated ED-Wsc1-Δ68-80
ED-Wsc1-Δ68-80
ED-Wsc1-Δ68-80
% Remaining
WT
Δpep4
Time of chase (min)
ED-Wsc1-Δ68-80
% Remaining
WT
Δcue1Δpep4
Δhrd1Δpep4
Time of chase (min)
ED-Wsc1-Δ68-80
% Remaining
WT
Δcue1
Δhrd1
Time of chase (min)
C
ED-Wsc1 Δ68-80Luminal
Time of chase (min)
0 30 60
WT
Δpep4
Δcue1
Δhrd1
ED-Wsc1-Δ68-80Luminal
% Remaining
WT
Δpep4
Time of chase (min)
ED-Wsc1-Δ68-80Luminal
% Remaining
WT
Δcue1
Δhrd1
Time of chase (min)

Figure S8